A U-shape fibre-optic pH sensor based on hydrogen bonding of ethyl cellulose with a sol-gel matrix

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Supplementary Material

Cytotoxicity testing

Bare silica optical fibres (Ø: 125 µm) and circular glass coverslips (Ø: 22 mm) were separately coated with the prepared film by dip coating for conducting direct contact and elution methods in cytotoxicity assays, respectively. The same drying conditions as described in sensor fabrication section were used and fibres and glass coverslips are sterilised with UV before incubation with culture media.

Cell Culture: NIH 3T3 mouse fibroblast cells were used (Public Health England, Cultures Collection, Porton Down, Salisbury, UK). Cells were grown to passage 9 before seeding. Cells were grown in growth medium consisting of DMEM (Sigma; Cat no: D6421) supplemented with 5ml 200mM L-glutamine (Sigma; Cat no: 59202C), 5ml penicillin/streptomycin solution (Sigma; Cat no: P4333) and 50ml heat inactivated foetal bovine serum (Sigma; Cat no: F9665). A well plate (Corning™ Costar™ 96-Well, Cell Culture-Treated, FlatBottom Microplate; Fisher; Cat no: 10695951), was used for both the direct contact and the elution trials. Wells were seeded at $4.8x10^3$ cells per well ($15x10^3$ cells per cm^2) cm^2 and allowed to grow in cell culture medium for 24h at 37°C + 5% CO_2.

Elution Method: 4 glass coated glass coverslips were placed inside a borosilicate glass vial along with 5 mL of growth medium (after incubation with the samples this is called eluate medium) for 24h at room temperature. This meets the requirement of ISO standard (BS EN ISO 10993-12:2012) in defining the minimum sample volume while extracting potential leaching from a sample. After 24h growth of cells, the growth medium was removed from the cells, and eluate media incubated with the different samples applied to each well. Samples were tested with 5 technical replicates. Cells were then left to grow further in the eluate media for either 24h or 72h. Normal culture medium was used as a control. After either 24h or 72h growth in the eluate media, a neutral red assay was carried out on the cells.

Direct Contact Method: The coated optical fibres were cut into 5mm lengths and transferred into 5 wells of a 96 well-plate (1 fibre per well). Wells were then seeded with cells and growth observed after 24h and 72h. Control samples were not exposed to fibres but were otherwise treated in the same way as cells exposed to fibres.

Neutral red cell cytotoxicity assay: Cell media was removed from the well plate containing cells grown as described for the elution or direct contact methods and replaced with 150µl of neutral red medium. Neutral red medium was prepared by the addition of neutral red stock (4mg/ml neutral red

dye (Sigma; Cat No: N4638) dissolved in 1ml deionised water) to culture medium in a ratio of 1:100 under sterile conditions. Cells were incubated for 2h at 37°C in an atmosphere with 5% CO_2. After incubation, unincorporated neutral red was removed by washing 3 times with phosphate buffered saline. 150 mL de-staining solution (50% v/v pure ethanol (Sigma; Cat No: 32205), 49% v/v deionised water and 1% v/v glacial acetic acid (Fisher Scientific; Cat No: 124040010)) was added to each well. The well plate was then placed on a plate shaker for 10 min at room temperature lysing the cells and dissolving the incorporated dye from the cells. The plates were read using the BioTek ELx800 UV plate reader (primary wavelength 490nm; reference wavelength 630nm).

Calculation attenuation coefficient γ as a function of n_2.

To obtain the correlation of attenuation coefficient γ to the refractive index n_2 by using Eq. (2), we take $\alpha=1/18\pi$ (< minimum complementary critical angle (7/100 π) when n_2 =1.42, in this case, the beam undergoes total internal reflection within the fibre) , the input wavelength $\lambda=0.6\,\mu m$, $n_1=1.458$, the absorption coefficient k=0.7. The refractive index of the film n_2 is varied from 1.33 (refractive index of the water) to 1.42 at a step of 0.01 for the simulations. The complementary critical angle (α_c) therefore changes with n_2.

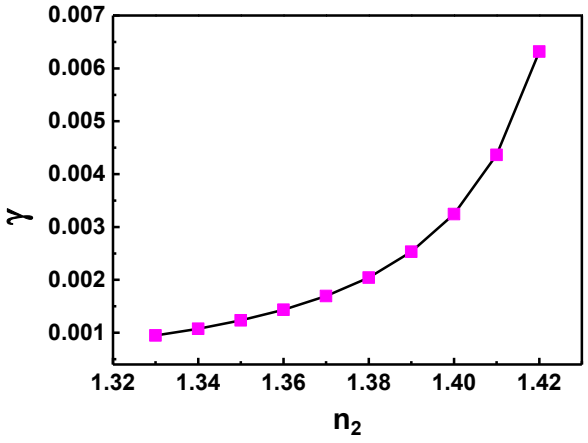


Figure.S.1 The relationship between the attenuation coefficient γ and the refractive index n_2 (From Eq. (2)).

Refractive index calibration of a bare U-shape sensor

To demonstrate the refractive index sensitivity of the U-shape optical fibre sensor**.** A U-shape sensor that is fabricated with the same fabrication method is subsequently immersed into solutions of different refractive index. Solutions with variable refractive index are generated by mixing a lower refractive index media (water, n=1.333) and higher refractive index media (isopropyl alcohol, n = 1.377) with different volume

ratios. Refractive index value of the prepared solutions is measured by a digital refractometer.

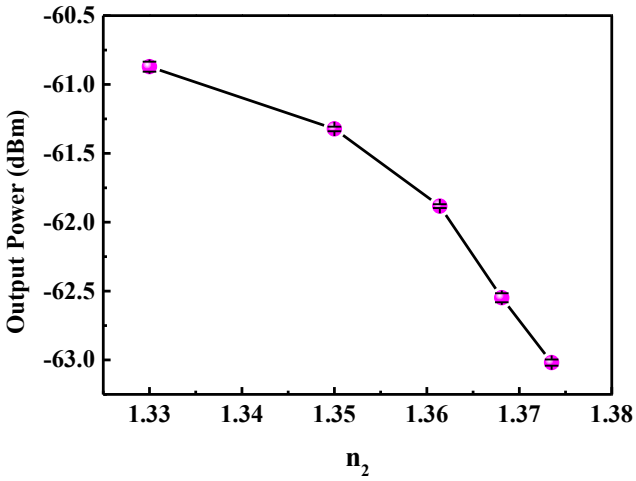


Figure.S.2 Experimental data from a bare U-shape fibre demonstrating that the output power reduces with the medium refractive index. Each point is the average of three times measurement, and error bar is smaller than marker size.

Absorption measurement of the pH film under different pH condition.

The absorption measurement of the pH sensitive film under different pH solutions is conducted by a UV-Vis absorption spectroscopy, in which the pH sensitive film is coated on a microscope glass slide and further immersed into different pH solutions for obtaining absorbance value at wavelength of 609 nm. The results in Fig.S.3 demonstrate that the absorbance value at the specific wavelength does not change as the pH value changes from 2.2 to 11.4. Considering the fact that the film thickness doesn't change during measurements, the absorption coefficient (k value in Eq.(2)) of the film remain constant under different pH solutions.

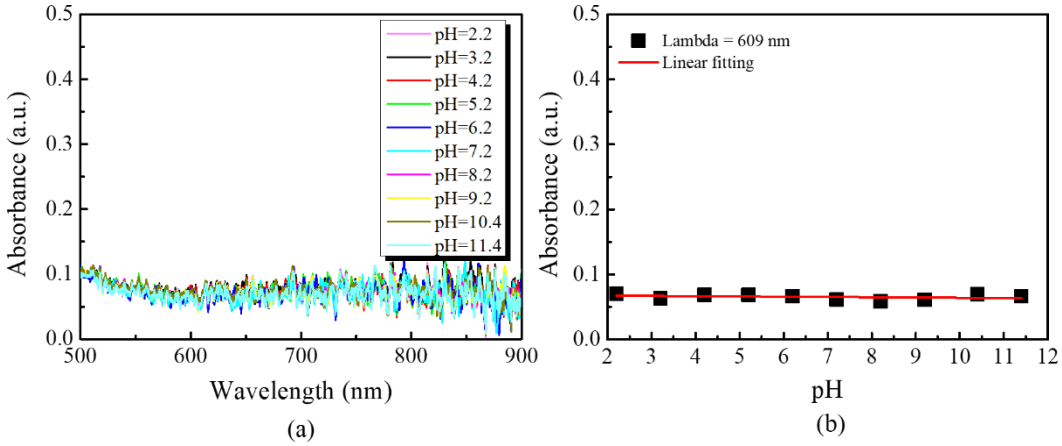


Figure.S.3 (a) The absorption spectra of the pH sensitive film under different pH solutions. (b) Absorbance value of the wavelength at λ=609 nm at different pH solutions

Figure.S.4

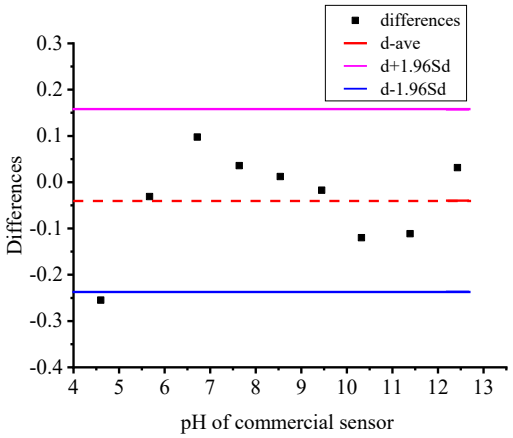
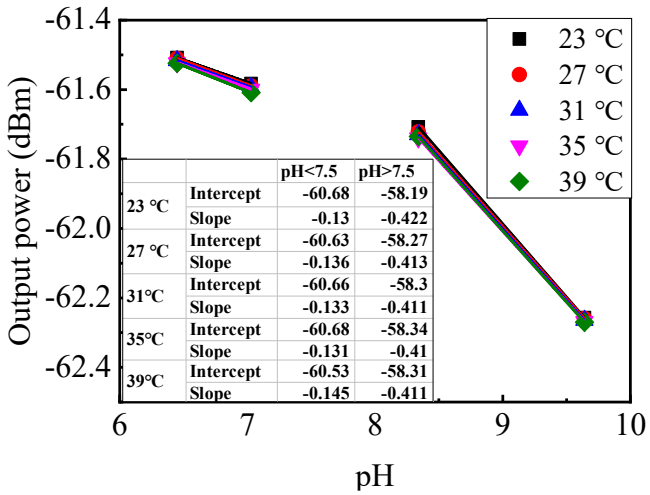


Figure.S.4 The Bland-Altman Plot of the measured pH values between the proposed U-shape sensor and the commercial sensor.

Figure.S.5



		pH<7.5	pH>7.5
23 ℃	Intercept	-60.68	-58.19
	Slope	-0.13	-0.422
27 ℃	Intercept	-60.63	-58.27
	Slope	-0.136	-0.413
31℃	Intercept	-60.66	-58.3
	Slope	-0.133	-0.411
35℃	Intercept	-60.68	-58.34
	Slope	-0.131	-0.41
39℃	Intercept	-60.53	-58.31
	Slope	-0.145	-0.411

Figure.S.5 The pH response of the sensor at different temperature.